



## The effect of oral contraceptive on the oral health with the evaluation of Salivary IgA and Streptococcus Mutans in some Iraqi women

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### Abstract

**Aim:** To assess if there is any effect of using oral contraceptive and the duration of using on the inflammatory responses of the gingival tissues, concentration of secretory immunoglobulin A ( IgA) and the colony count of mutans StreptococcusMS in saliva .

**Methods:** This study includes 80 women in their reproductive age (16-40) year. 40 women who were using oral contraceptive in different duration, as the study group and 40 women who not used oral contraceptive and it were assigned to the control group. Both groups were clinically examined (for dental caries using DMFS Index, and gingival condition using gingival index GI of Loe&Silness ) and laboratory assessment (by using non stimulated saliva for each woman was collected and homogenized then Mutans Streptococci (MS) were counted ,isolated, purified and diagnosed according to morphological characteristic and biochemical tests also, the Salivary IgA concentration was measured by using single radial immune diffusion method in different duration of oral contraceptive using ).

**Results:** Gingival index was significantly higher among oral contraceptive users than non-users ( $P<0.01$ ), which was correlated with the duration of usage ( $r =0.50$ ).sIgA concentration higher in study group than in control group ( $P<0.01$ ) which also increased positively with duration of using( $r=0.80$ ). A positive correlation between sIgA and gingivitis ( $r=0.54$ ) was found. DMFS Index and the number of MS colony were highly significant in study group than the control ( $P<0.01$ ). MS colony count was positive correlated with duration of usage ( $r=0.88$ ) but the DMFS had too weak correlation with the duration of using which was a non significant at ( $P>0.05$ ).

**Conclusion:** The use of contraceptive pills for a long period increases the likelihood of gingival inflammation and significantly associated with increase sIgA level, so the regular use of oral contraceptive seems to enhance mucosal immune function in study subjects.

**Key words:** Oral contraceptives, Gingivitis, Secretary Immunoglobulin A (sIgA), Dental caries.

### Introduction

Oral contraceptives have become a widely used form of birth control. Oral contraceptives are used for pregnancy prevention, treatment of menstrual irregularities and endometriosis. It prevents pregnancy primarily by inhibiting ovulation through the combined actions of progestin and estrogen. Progestin inhibits ovulation by suppressing the cyclical release of luteinizing hormone (LH) from the anterior pituitary gland. Progestins also create thick cervical mucus that slows sperm transport and inhibits capacitation (the activation of enzymes that permit the sperm to penetrate the ovum) <sup>(1,2)</sup>. Estrogen contributes to ovulation inhibition by suppressing the release of follicle-stimulating hormone (FSH) and LH. Estrogen also accelerates ovum transport, which decreases fertilization time. Finally, estrogen alters secretions within the uterus to produce areas of edema and dense cellularity, making implantation less likely<sup>(3)</sup>. Since all oral contraceptives act by artificially altering sex hormones level, their influence on gingival inflammation has been observed. A rise in the hormones, estrogen and progesterone, increases the risk of inflammation of the gingiva <sup>(4)</sup>. Progesterone and estrogen stimulate key factors involved in the inflammatory response. They stimulate bacterial growth in the mouth, resulting in a shift in bacteria flora <sup>(5)</sup>.

Gingival changes include inflammation and enlargement with increased amount of fluid flow into the tissue. Hence, it is expected that the same gingival changes seen during pregnancy will also be seen in women taking oral contraceptives<sup>(6)</sup>. Clinical studies reported higher prevalence's of gingival inflammation, periodontal attachment loss, and gingival enlargement in women taking oral contraceptive medication compared with those who were not <sup>(7,8,9)</sup>.

Secretary immunoglobulin A (SIgA) is the main immunoglobulin found in saliva and other body secretions. Biologically, sIgA provides the first line of immune defense in the oral environment <sup>(10)</sup>. It is responsible for inhibiting the bacterial adhesion on the enamel and epithelial cells, acting in synergy with other defense mechanisms, making inactive bacterial enzymes and toxins and activating the complement. It is partially involved in cell-mediated immune responses. Thus, sIgA limits the invasion of various antigens in the mucosal epithelium and is involved in the maintenance of bacterial environment in the mouth and in the formation of bio films on the enamel surface <sup>(11)</sup>.

The present study was designed to determine if current use of oral contraceptives intensify gingival disease, the concentration sIgA and the count of mutans Streptococcus in different duration of using also to can be determined the correlation of sIgA concentration with the gingival diseases.

## Materials and Methods

### A- Subjects:

This prospective cross sectional study was done from February, 2012 to October ,2012 ,80 women ranged from 16 to 40 years was included in the study sample were taken from 100 women attending to hospital of dentistry college of Al-Mustansiriya University for dental consultation selected by random sampling, then we asked them about history of using oral contraceptive for control of birth and duration of use and categorized them to two groups, user to be the study group(40) and non-user(40) as control group ,after that we examined them to see if they had gingivitis by using the gingival index as diagnostic parameter and a laboratory testing

done for assessing the level of IgA and streptococcal mutans count in saliva for both groups. All women enrolled in this study were met the criteria for this study (by questionnaires); where they are non-smokers, not using antibiotics, married and had no significant medical history such as diabetes mellitus.

#### **B- Dental examination:**

A clinical examination had been performed by using DMFS index 1989<sup>(12)</sup> and Gingival health status of the women was determined by recording the oral Gingival index (GI of Loe & SiIness1963) using the classical methodology of the index determination <sup>(13)</sup>. The examinations were carried out on dental chair by using of sterilized examination sets including dental mirrors, explorers and periodontal prop. Non stimulated saliva for each woman was collected in sterilized capped bottles. Salivary samples were collected under conditions according to Dansanyake et al 1995<sup>(14)</sup>.

#### **C- Isolation of Microorganisms:**

The identification of isolated microorganism was depending on morphological examination of microbial cell (by using Gram stain method) and culture characteristics on culture media: Brain Heart Infusion Agar (BHI-A) and Mannitol Sorbitol Fuchsin Agar (MSF-A)<sup>(15)</sup>. According to (Al-Mizraqchi, 1998)<sup>(16)</sup> and by using:

1. Catalase production test
2. Carbohydrate fermentation test by using mannitol fermentation

#### **D- Salivary IgA evaluation:**

Salivary IgA concentration was quantities by using radial immune diffusion method.

#### **E- Statistical analysis**

The following statistical data analysis approaches were used in order to analyze and assess the results of the study:

#### **1. Descriptive data analysis:**

(Standard Deviation, Standard Error, (95%) Confidence interval for population Mean of Score values and Correlation ship (person – coefficient).

#### **2. Inferential data analysis:**

These were used to accept or reject the statistical hypotheses, which included the following: Levene test for testing the homogeneity of variances for equality of variances and student t-test for equality of Means (testing of coincidence), Estimation the determination coefficient in the Linear regression analysis model. Simple linear regression parameters estimates and testing their significance as well as applying ANOVA for regression models.

## **Results**

The results of the study show that the irrespective of contraceptive usage duration, Age did not show any statistically significant difference ( $P > 0.05$ ) for the observed frequency's distributions of the two samples, (Study and Control) which were corresponding proportionally. In other words, we can conclude that these characteristic variables doesn't reported any source of variation between the two groups and that established /or adapted the two independent samples for studying the phenomena (Table 1). The results of testing coincidence between the study and control groups indicating that a highly significant different at  $P < 0.01$  were obtained at the different of the studied parameters and that interpreted the complication of using oral contraceptive (Table 2 and 3).

The GI and DMFS Index of OC users were significantly higher than those of non-users. The slope value indicating that with increasing one unit of scale in the (duration per months), a positive increment should be occurred in the unit of the causes gingivitis estimated with (0.05554) and that increment recorded a highly significant effect at  $P < 0.01$  (Table 4). While too weak correlation ship between DMFS and duration neither by linear association nor non-linear forms (models) with a non significance at  $p > 0.05$ .

Table (5) illustrate that The slope value indicating that with increasing one unit of scale in the (duration per months) , a positive increment should be occurred in the number of colony of MS and estimated with (7.11011) and that increment recorded too highly significant effect at  $P < 0.0001$  . For the SIgA, increasing one unit of scale in the (duration months), a positive increment should be occurred estimated with (1.10545) and that increment recorded too highly significant effect at  $P < 0.0001$  (Table 6). Table(7) showed that a meaningful linear regression tested in two tailed alternative of the statistical hypothesis between the GI and s IgA Increasing one unit of S IgA , a positive increment should be occurred in the unit of the causes gingivitis and estimated with (6.774415) and that increment recorded too highly significant effect at  $P < 0.0001$  .

Figure (1, 2 and 3) showed the long term trend of the cause's correlation ship between the duration of using oral contraceptive and GI, MS colony count and SIgA respectively. Figure (4) the co relationship between the GI and sIgA as a dependent variable.

## Discussion

The present study revealed that the rates of caries and gingivitis were significantly higher in women who using OC than in none using. Many studies have shown that the oral mucosa and salivary glands are sensitive to estrogen action. However, the expression of estrogen receptors (ERs) within these tissues is an area of controversy. ERs exist as two subtypes (ERalpha and ERbeta). ERalpha was not detected in oral buccal and gingival epithelium or in salivary glands. In contrast, ERbeta was widely expressed at high levels in all oral tissues. Importantly, these results suggest that estrogens may act via ERbeta in oral tissues and explain the effect of hormonal changes on the oral mucosa as well as on saliva secretion and composition<sup>(17)</sup>.

The level of progesterone, increase the blood flow to the gum tissue and causes gums to be more sensitive and vulnerable to irritation and swelling. The gingival tissue responds to increased levels of estrogen and progesterone by undergoing vasodilatation and increased capillary permeability<sup>(18)</sup>. Consequently, there is an increased migration of fluid and white blood cells out of blood vessels. Also associated with increased progesterone levels are alterations in the existing microbial populations. The levels of Gram-negative anaerobic bacteria, such as *Prevotella intermedia*, increase as a result of the high concentration of hormones available as a nutrient for growth<sup>(19)</sup>.

Increases in the rate of both estrogen metabolism by gingiva and in the synthesis of prostaglandins were found to contribute to the gingival changes. Alteration in progesterone and estrogen levels have been shown to affect the immune system and both the rate and pattern of collagen production in the gingival. Both of these conditions reduce the body's

ability to repair and maintain gingival tissues<sup>(6, 20)</sup>. It has been noted that the clinical signs of diseases seen include redness, swelling, and bleeding from the gingiva. Such findings suggest the existence of relationship which is in agreement with those of many reports<sup>(8,9,21,22)</sup>. Despite numerous studies evaluating the improved safety of current oral contraceptive formulations in other body systems<sup>(23,24)</sup>.

The prevalence of dental caries and the number of colonies of Mutans Streptococci were higher in women using OC than in non-using. MS colony was increased positively with the increasing in duration of usage, but the DMFS had no correlation ship with the duration. Our findings are in disagreement with other studies which found no significant variation between groups<sup>(25)</sup>. Kornman and Loeshe reported that one-fourth of the women of reproductive age had dental caries, a disease in which dietary carbohydrate is fermented by oral bacteria into acid that de-mineralizes enamel<sup>(26)</sup>.

Secretary immunoglobulin A (SIgA) constitutes the predominant immunoglobulin isotype in secretions, including saliva. Secretary Immunoglobulin A is a member of the adaptive immune response and is the predominant immunoglobulin of the mucosal immune system. It is considered to be the first line of defense of the host against pathogens which colonize or invade surfaces bathed by external secretions<sup>(27, 28)</sup>. The main function of SIgA antibodies seems to be to limit microbial adherence as well as penetration of foreign antigens into the mucosa. Naturally occurring SIgA antibodies reactive with a variety of indigenous bacteria have been detected in saliva. Furthermore, indigenous bacteria of the oral cavity have been found to be coated with SIgA<sup>(29, 30)</sup>.

The results of the present study revealed an increase in the level of SIgA in saliva of women using OC compared to the control group and this level was elevated with increasing the time of using. The study also revealed an association between the SIgA level and gingival disease which was highly significant.

This is because what seems to stimulate the secretion of immunoglobulin A into the saliva is the extension of the inflamed gingival area and severity of local and restricted areas of inflammation. An explanation for this, that inflammation can stimulate the production and secretion of IgA. Thus, a larger number of inflamed gingival areas, in addition to stimulating a greater production of IgA<sup>(31, 32)</sup>. SIgA may also increase the possibility of serum IgA having access to saliva either through gingival fluid or through the damaged mucosal surface itself. The increased release of serum IgA due to an increased permeability of the crevicular epithelium during gingival inflammation may contribute to the rise in IgA levels observed in saliva. However, higher SIgA levels have also been observed in parotid saliva of subjects with gingival inflammation<sup>(33, 34)</sup>.

Little information exists on the role of salivary IgA in the development of gingivitis and periodontitis. Salivary IgA could control sub gingival plaque, since secretory IgA antibodies do not penetrate the crevice or pocket. It is possible that salivary IgA antibodies, by modulating the accumulation of supragingival plaque, control the formation and composition of sub gingival plaque and its potential for causing disease. It could limit the spread of the disease by preventing bacterial transmission from infected to healthy gingival sites<sup>(35)</sup>. Past studies have reported positive<sup>(36, 37,38)</sup> or

no significant (39,40,41) correlations between total salivary IgA levels and gingivitis.

## Conclusion

As with pregnancy-associated gingivitis, gingival inflammation in women on oral contraceptives occurs in the presence of very little plaque. The most profound gingival changes are seen in the first few months after starting the contraceptive and its severity increased with time. If the condition worsens, a different formulation may be tried. Once the woman discontinues the contraceptive, the gingival condition will reverse.

**Recommendation:** this study recommended to not use or reduce using of oral contraceptive and suggests studying the differences of the immune cells and cytokines levels both users and non users.

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Table (1): Distribution of age groups between the two independent samples with comparison significant.

C.S. P-value	Cumulative Percent	Percent	Frequency	Age Groups	Groups
X <sup>2</sup> = 5.309 P = 0.150 NS	10	10	4	15 - 19	Study(users)
	35	25	10	20 - 24	
	85	50	20	30 - 34	
	100	15	6	35 - 39	
	-	100	40	Total	
	29.15± 5.83				Mean ± SD
	5	5	2	15 - 19	Control(none user)
	60	55	22	20 - 24	
	90	30	12	30 - 34	
	100	10	4	35 - 39	
	-	100	40	Total	
	28.45± 3.75				Mean ± SD

Table (2): The mean and SD of the parameters at the study and control groups (users and none users) woman.

Max.	Min.	95% C. I. for Mean		Std. E.	Std. D.	Mean	No.	Groups	Parameter
		U.b.	L.b.						
2.7	1.1	2.03	1.72	0.07	0.47	1.88	40	Study(users)	GI
0.9	0.2	0.60	0.37	0.06	0.25	0.48	40	Control(none users)	
34	2	15.99	11.21	1.18	7.46	13.60	40	Study(users)	DMFS
14	3	11.15	7.25	0.93	4.18	9.20	40	Control (none users)	
160	18	59.74	37.56	5.48	34.68	48.65	40	Study (users)	MS(colony)
11	4	8.31	6.09	0.53	2.38	7.20	40	Control (none users)	
34.2	11.5	19.19	15.43	0.93	5.89	17.31	40	Study (users)	SIgA mg/dl
0.6	0.2	0.47	0.33	0.03	0.15	0.40	40	Control (none users)	

Table (3): Comparison significant for of the studied parameters between the users and none users groups .

C.S. P-value	Equality of means test t- test			Equality of variances test Levene's test		Study Parameters
	P-value	Statistic	d.f.	P-value	Statistic	
HS	0.000	57.859	15.005	0.000	14.152	GI
HS	0.005	57.137	2.925	0.046	4.162	DMFS
HS	0.000	39.728	7.523	0.000	24.038	MS(colony)
HS	0.000	39.095	18.148	0.000	21.034	SIgA mg/dl

HS: Highly Sig. at P&lt;0.01

Table (4): Simple correlation analysis for the effectiveness of long duration of using oral contraceptive and causes gingivitis

GI Dependent variable.. Method.. Linear in the					
List wise Deletion of Missing Data					
Meaningful Linear regression Tested in two tailed alternative Statistical hypothesis			0.50405	Correlation R	
			0.25407	R Square	
			0.23444	Adjusted R Square	
			0.41456	Standard Error	
0.0009			Sign. F =	12.943	F =
Variables in the Equation					
Sig. of ( t )	( t )	Beta	SE.B	B	Variable
0.0009	3.598	0.504054	0,015438	0.05554	Duration
0.0000	10.736	-	0.135070	1.45012	(Constant)

Table ( 5 ) : Simple correlation analysis for the effectiveness of long duration of using oral contraceptive and MS. colony

MS.COLONY Dependent variable.. Method.. Linear in the					
List wise Deletion of Missing Data					
Meaningful Linear regression Tested in two tailed alternative Statistical hypothesis			0.88151	Correlation R	
			0.77706	R Square	
			0.77119	Adjusted R Square	
			16.59015	Standard Error	
0.0000			Sign. F =	132.449	F =
Variables in the Equation					
Sig. of ( t )	( t )	Beta	SE.B	B	Variable
0.0000	11.509	0.881509	0.617807	7.110110	Duration
0.2948	-1.062	-	5.405367	-5.74234	(Constant)

Table ( 6 ) : Simple correlation analysis for the effectiveness of long duration of using oral contraceptive and SIgA

SIgA Dependent variable.. Method.. Linear in the					
List wise Deletion of Missing Data					
Meaningful Linear regression Tested in two tailed alternative Statistical hypothesis			0.80711	Correlation R	
			0.65143	R Square	
			0.64226	Adjusted R Square	
			3.52255	Standard Error	
0.0000			Sign. F =	71.0165	F =
Variables in the Equation					
Sig. of ( t )	( t )	Beta	SE.B	B	Variable
0.0000	8.427	0.807112	0.131178	1.10545	Duration
0.0000	7.714	-	1.147710	8.853307	(Constant)

Table ( 7 ) : Simple correlation analysis for the effectiveness of GI and SIgA

SIgA Dependent variable.. Method.. Linear in the					
List wise Deletion of Missing Data					
Meaningful Linear regression Tested in two tailed alternative Statistical hypothesis				0.54500	Correlation R
				0.29703	R Square
				0.27853	Adjusted R Square
				002435.	Standard Error
0.0003	Sign. F =		16.05598	F =	
Variables in the Equation					
Sig. of ( t )	( t )	Beta	SE.B	B	Variable
0.0003	4.007	0.54500	1.690649	6.774415	GI.
0.1666	1.410	-	3.267154	4.607973	(Constant)

Table (8): The correlation between the effectiveness of the duration of using oral contraceptive and different study parameters .

C.S .value	P	P .value	Correlation	Parameters under correlations
HS		0.0009	R=0.50	GI with duration
NS		0.805	R=0.04	DMFS with duration
HS		0.00000	R=0.88	MS colony with duration
HS		0.000	R=0.80	SIgA with duration
HS		0.0003	R=0.54	GI with sIgA

Correlation is significant at the 0.01 level

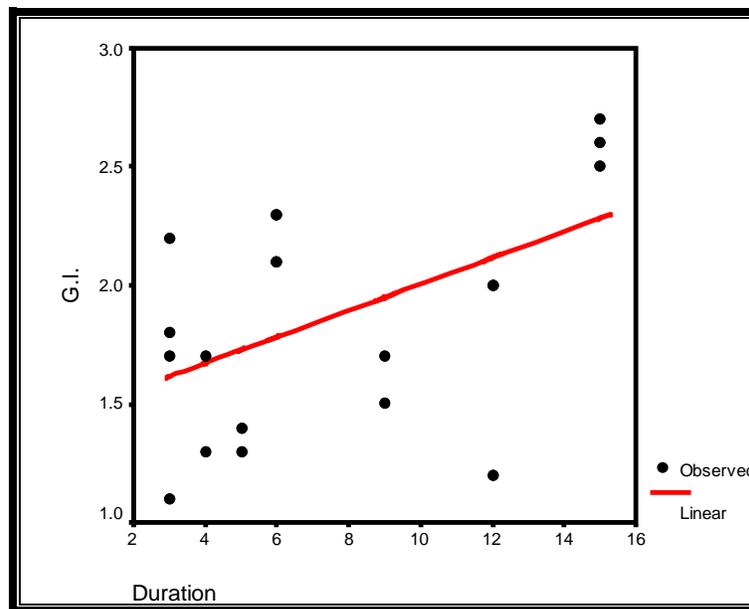


Figure (1): Scatter diagram of the effectiveness of the duration of using oral contraceptive and causes gingivitis

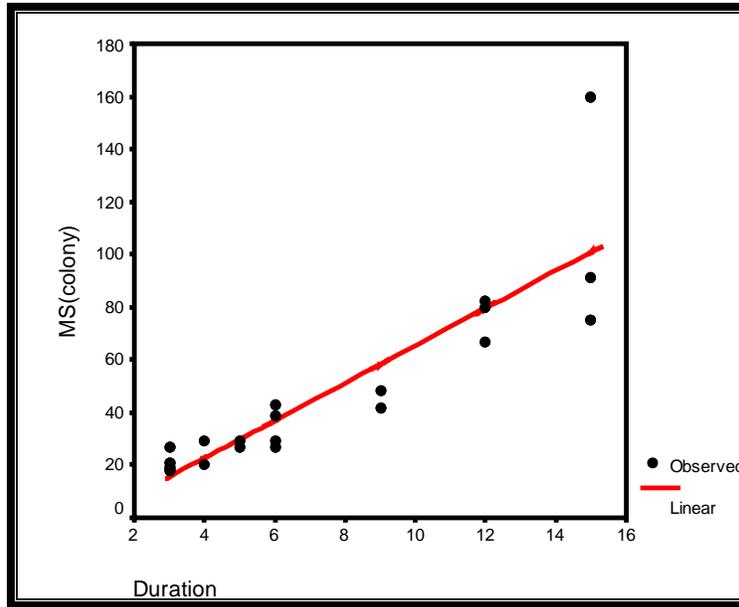


Figure (2): Scatter diagram of the effectiveness of the duration of using oral contraceptive and MS. Colony count .

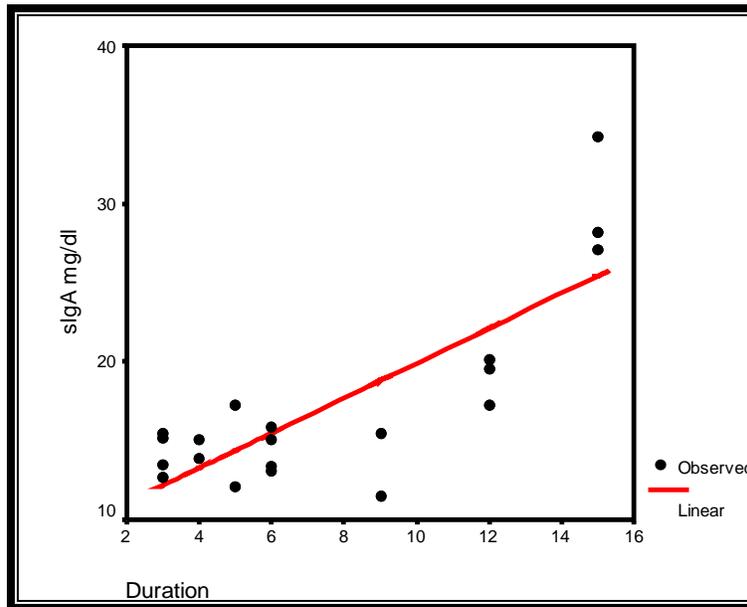


Figure (3):- scatter diagram of the effectiveness of the duration of using oral contraceptive and SIgA .

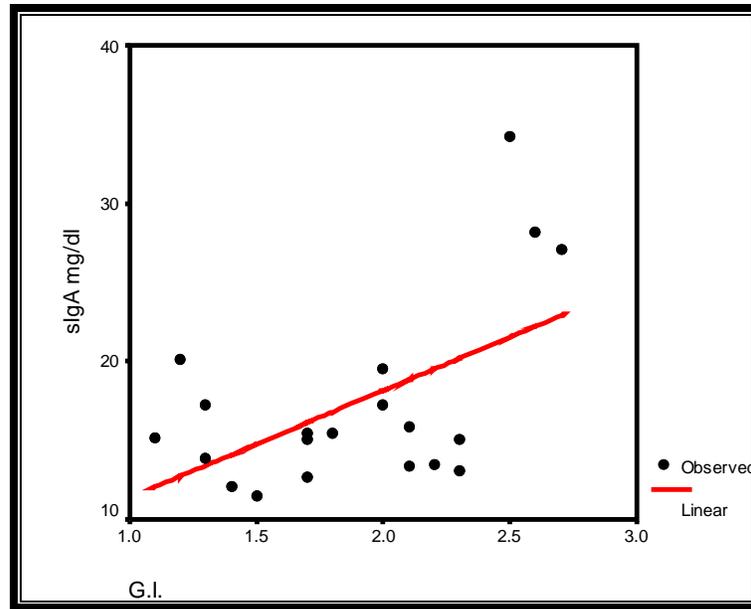


Figure (4): The scatter diagram of the effectiveness of the G.I. for SIgA .